# Rational Development of a Soluble Prodrug of a Cytotoxic Nucleoside: Preparation and Properties of Arabinosyladenine 5′-Formate

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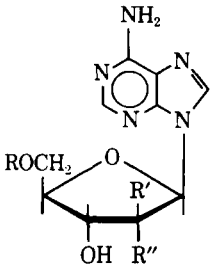
**Abstract** □ The 5′-*O*-formate ester of arabinosyladenine was synthesized and shown to be suitable as a water-soluble prodrug, being at least 60 times more soluble than the parent cytotoxic nucleoside. This increased solubility was ascribed to a decrease in intermolecular interaction in the crystalline state, as evidenced by an ~90° lower melting point for the ester relative to the parent compound. The prodrug reverted to the parent compound in aqueous solution, its pH–rate profile being V-shaped with maximum stability at a pH ~4.2, corresponding to a half-life of about 10 days. The rate of hydrolysis of the prodrug at 37° in 91% human serum and 91% whole blood was studied. At an initial concentration of 0.4 mg of prodrug/ml of 91% whole blood, reversion to arabinosyladenine appeared to be essentially complete in about 15 min. The prodrug did not appear to be subject to enzymatic deamination. This feature, together with the good solubility of the prodrug, makes possible the effective formulation of arabinosyladenine for intravenous purposes. The rationale involved in the general design of a prodrug and the specific considerations necessitated in the case of adenine arabinoside are discussed.

**Keyphrases** □ Arabinosyladenine 5′-formate—synthesized as highly water-soluble prodrug, hydrolysis rates in biological fluids □ Prodrugs—synthesis of highly water-soluble arabinosyladenine 5′-formate, hydrolysis rates in biological fluids □ Cytotoxic nucleosides—synthesis of arabinosyladenine 5′-formate as highly water-soluble prodrug, hydrolysis rates in biological fluids

The nucleoside arabinosyladenine[1] (I) is an epimer of adenosine (II) and has been shown to be an effective agent in the inhibition of the growth of viruses and tumors in various *in vitro* and *in vivo* tests (1–6). The low water solubility[2] (0.0018 *M*, 0.5 mg/ml) of this drug and the large (2.5 g) human doses suggested have hampered its clinical testing[3]. Infusions of several liters of intravenous vehicle containing the dissolved drug have been employed in some cases[3]. The present studies were undertaken to develop a more soluble prodrug form of I and thus reduce the minimum volume in which this dose (2.5 g) could be dissolved.




I: R = R″ = H, R′ = OH
II: R = R′ = H, R″ = OH
III: R = HCO, R′ = OH, R″ = H

## DISCUSSION

A comparison of the chemical structures of I and II shows that they differ only in their stereochemical configuration at the 2′-position of the sugar group. Both substances appear to be quite polar, and they might be expected to be similarly solvated in aqueous solution. The lower solubility of I ($1.8 \times 10^{-3}$ *M*) relative to II (~0.02 *M*)[2] may be explained by stronger intermolecular hydrogen bonding in the crystal lattice of I due to the different geometry. This rationalization appears to be supported by the observed differences in melting points (I, mp 258–260°; II, mp 235°), where the higher melting material is less soluble.

One method of achieving an apparent increase in the solubility of I is through the use of a water-soluble prodrug form (7, 8) of I. This method involves the identification of a suitable chemical derivative of I that reverts to I under *in vivo* conditions. Two different approaches for increasing solubility through prodrug formation are immediately apparent. The first is to prepare a derivative considerably more hydrophilic than I, in which case the increase in solubility would be achieved through increased solvent–solute interactions. The 5′-phosphate of I (9), which is simply an epimer of adenosine monophosphate, is an example of this type of prodrug and has already been reported.

The second type of prodrug with increased solubility relies on the perturbation of the geometry of the crystal and/or a decrease in the capacity for intermolecular interaction to achieve the increase in solubility. Such changes would be expected to increase the free energy of the crystalline solute and thereby would tend to increase solubility. An example of this approach is the $N^6$-(dimethylamino)methylene derivative of I (10).

Although the specific prodrug examples cited successfully achieved significant increases in solubility, other criteria, such as the rate of release of the drug from the prodrug form, also should be considered in the design of a prodrug.

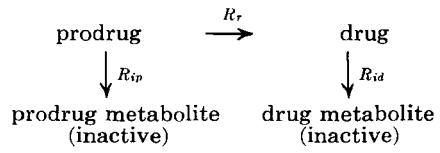
If the purpose of utilizing the prodrug approach is to attain improved drug delivery as evidenced by greater concentrations of the drug in the body fluids, then it is the position of these investigators that the prodrug should not persist appreciably once the delivery function has been achieved. Therefore, unless designed specifically for other purposes such as timed release, prodrugs should be rapidly converted to the drug itself in the relevant biological medium. If such is not the case and the drug is released over a period of hours, some doubt will exist as to whether the nature of the observed pharmacological response is due to the drug or to the prodrug. This is particularly important in cases where the prodrug itself, because of inherent chemical or structural features, may be likely to exert a biological response.

The rate of regeneration of a drug from its prodrug form may also be important from a kinetic standpoint when attempting to achieve increased blood levels of drug. A simplified representation

[1] Arabinosyladenine is referred to in a number of ways in the literature including 9-β-D-arabinofuranosyladenine, ara-A, NSC-404241, and adenine arabinoside.

[2] Determined in these laboratories.

[3] J. P. Davignon, National Cancer Institute, personal communication.




*Scheme I*

of potential problems of the kinetic type are presented in Scheme I, where $R_{ip}$ and $R_{id}$ represent the rates of inactivation of the prodrug and the drug, respectively, and $R_r$ represents the rate of release of the drug from the prodrug. From an inspection of Scheme I, it is obvious that the concentration of the drug that can be attained is dependent on the magnitude of $R_r$ relative to $R_{ip}$ and $R_{id}$. As the magnitude of the ratio $R_r/[R_{ip} + R_{id}]$ increases, the concentration of the drug that can be achieved is increased.

When one considers that arabinosyladenine undergoes enzymatic deamination (11, 12) to the corresponding biologically inactive hypoxanthine (6) rather rapidly[4], it is obvious that a slow rate of reversion, such as that exhibited by the previously reported prodrugs of I (9, 10), will result in extremely low concentrations of I *in vivo*. Hence, an important consideration in this work was the identification of a prodrug that, *in vivo*, would rapidly revert to I as well as provide the desired solubility properties.

The chosen approach for increasing the solubility of I involved the formation of a prodrug exhibiting decreased intermolecular hydrogen bonding in the crystalline state and thereby increasing the activity of the solid. It was anticipated that this objective could be achieved by reducing the hydrogen donor capacity of the sugar portion of the molecule. The replacement of the hydrogen atom of a hydroxy group by acylation or alkylation was expected to accomplish this goal. Again, any such derivative should rapidly revert to I under *in vivo* conditions. The well-known facts that esters are normally more rapidly cleaved than are ethers and that blood contains esterases suggested that an ester would be preferred over an ether as a prodrug.

Esterification of the 5′-hydroxy group was decided upon, since it was expected to offer some advantages. First, the 5′-hydroxy is a primary alcohol, while the 2′- and 3′-hydroxy groups are secondary alcohols. Since it was shown (13) with a series of butyl and propyl esters that the esters of primary alcohols are normally cleaved more readily than the corresponding esters of secondary alcohols, an ester at the 5′-position would be expected to be the most rapidly hydrolyzed of the possible esters of I.

Second, earlier work (6, 11) on adenosine and related compounds strongly suggested that a free hydroxy group at the 5′-position was required to serve as a good substrate for adenosine deaminase. Therefore, derivatization at the 5′-hydroxy would be expected to prevent or retard the enzymatic deamination of III as discussed previously. Such protection from deamination, if realized, would then assure that reversion of the prodrug would occur prior to any deamination.

The specific choice of the formyl group as the acyl group of choice for esterification of the 5′-position was based on the greater facility with which formate esters are hydrolyzed in comparison to more bulky homologs (14, 15) such as acetate and propionate. Another consideration in this choice was that the formyl group, being extremely compact, would not be expected to introduce any appreciable lipophilic character into the otherwise polar nature of I. On the basis of these considerations, arabinosyladenine 5′-formate[5] (III) appeared to be an attractive candidate as a prodrug of I.

## EXPERIMENTAL

Anhydrous I[6], mp 258–260°, was used as supplied. All other chemicals used were of the highest commercial grade available. The water was triply distilled, with the final distillation being from acid permanganate solution in an all-glass still.

**Liquid Chromatography**—High-pressure liquid chromatography (HPLC) was used for monitoring and optimizing the conditions for the synthesis of III and for following the kinetics of hydrolysis of III. Except for the eluants, the chromatographic systems used were identical for both purposes. The liquid chromatograph[7] was equipped with a UV (254 nm) detector and a 0.5-m stainless steel column having an internal diameter of 2 mm. The stationary phase was a strong cation-exchange resin[8]. Samples were eluted at 600 psig, which resulted in a flow rate of about 1 ml/min.

Since the chromatograph used was not insulated or thermostated, column temperatures during operation were subject to fluctuations in room temperature. The temperature varied from day to day but was in the range of 23–27°; fluctuations within the time period of a given run or series of runs were normally <1.5°. Such temperature variations caused retention times to vary by as much as 20–30%; peak heights were also found to vary. Because of such variation in peak heights, integration of the area of the peaks was performed using a planimeter. This approach yielded results that, when normalized as described later, were not noticeably affected by the temperature variation. Detailed differences between the HPLC systems used will be discussed.

*System Used in Following the Synthesis of III*—The eluant system used for monitoring and optimizing the synthesis of III was an aqueous solution containing 0.04 *M* $NaH_2PO_4$ and 0.05 *M* $NaNO_3$, pH 4.5. This system resulted in the separation of several components. Typical retention times were as follows: I = 1 min, III = 2 min, II (adenine)[9] = 2.5 min, arabinosyladenine 3′-formate[10] (IV) = 4 min, and arabinosyladenine 3′,5′-diformate[11] (V) = 12.5 min.

*System Used in Following Hydrolysis of III*—In studies on the hydrolysis of III, the eluant used was aqueous 0.04 *M* $NaH_2PO_4$, which had been adjusted to pH 4.2 with 10% $H_3PO_4$ in water. Although retention times varied somewhat with temperature fluctuations, typical values for the retention times of I and III were 1.5 and 4.5 min, respectively.

**Preparation, Isolation, and Characterization of III**—The synthetic method and conditions reported here were arrived at after monitoring the reaction by liquid chromatography and attempting to optimize the conditions with respect to the yield of III.

Compound I (10.0 g) was dissolved in anhydrous formic acid (16.0 ml) and maintained at 5° for 96 hr. The isolation of III was accomplished by the use of conventional column chromatography according to the following procedure.

The reaction mixture (after 96 hr) was diluted to 2 liters with ether while being stirred vigorously with a magnetic stirrer. The crystalline solid that separated was allowed to settle, and the supernate was decanted. The precipitate was washed with an additional 500 ml of ether, which was decanted. The ether solutions were combined (2.5 liters) and passed through a chromatographic column (7 cm i.d.) previously packed with an ether slurry of silica gel[12] (450 g). The solid residue remaining after the washing procedure was dissolved in acetone–ether (4:1, 3 liters) and passed through the column. Further elution with 2 liters of acetone–ether (9:1) followed.

These eluate fractions were found by HPLC analysis to contain little of the desired product and were discarded. Further elution with acetone (8 liters) was carried out and the eluate was concentrated *in vacuo* at room temperature. The gummy residue obtained was transferred to a crystallizing dish, using a minimal amount of acetone. A crystalline solid formed spontaneously on standing. The filtered and dried product (5.5 g, 50% yield) was found by HPLC analysis to contain >96% of III with the remaining 4% consisting of unreacted I and some II, IV, and V. Recrystallization from acetone resulted in a solid, mp 168–170°, which appeared to be essentially homogeneous by HPLC and TLC [silica gel, chloroform–methanol (2:1), $R_f$ 0.4]. This material was charac-

[4] Dr. Florence White of the National Cancer Institute stated, in a personal communication, that the deamination resulted in loss of antitumor activity and that the biological half-life for the enzymatic deamination of I was ~30 min.

[5] The prodrug, arabinosyladenine 5-formate, was recently assigned the identification number NSC 171240 by the National Cancer Institute.

[6] Furnished by the National Cancer Institute.

[7] DuPont model 840.

[8] SCX on Zipax, DuPont.

[9] Compounds II and III were not completely resolved, but the presence of small amounts of II was apparent as a small shoulder or peak on the much larger III peak. The retention time was confirmed with authentic II.

[10] Compound IV was not completely characterized but was tentatively identified as the 3′-formate ester of I.

[11] Compound V was isolated, characterized, and definitely established to be the 3′,5′-diformate ester of I (mp 153–155°).

[12] Silicar CC-7, Mallinckrodt.

terized as arabinosyladenine 5′-formate (III); IR (KBr): 1730 (s, C=O) $cm^{-1}$; NMR (dimethyl sulfoxide-$d_6$): $\delta$ 8.25 (s, 1, 5′-$O_2CH$), 8.16 (s, 2, 2- and 8-H), 7.35–7.05 (m, 2, N—H), 6.36 (d, $J$ = 4 Hz, 1, 1′-H), 5.95–5.55 (m, 2, 3′- and 2′-O—H), 4.55–4.35 (m, 2, 5′-H), and 4.35–3.85 (m, 3, 4′-, 3′-, and 2′-H) ppm.

Elemental analysis for carbon, hydrogen, and nitrogen was within 0.3% of theory for $C_{11}H_{13}N_5O_5$.

**Preparation of Whole Blood and Serum Samples**—Serum was prepared by inducing rapid and complete clotting of the blood samples through agitation with glass rods. The resulting sample was centrifuged and the supernatant serum was collected. When whole blood was desired, potassium oxalate (~2 mg/ml of blood) was added to the freshly drawn sample to prevent coagulation.

**Kinetic Studies**—*Hydrolysis in Aqueous Solutions*—Reactions were initiated by adding freshly prepared aqueous solutions of III to the buffered media in small screw-capped vials. For studies carried out at 25°, the solutions were preequilibrated in a water bath[13]; sampling was begun immediately after mixing. For studies at elevated temperatures, samples were mixed at room temperature and subsequently placed in the thermostated bath; no sampling was done for at least 15 min. Initial concentrations of III were approximately 1 mg/ml (~0.003 $M$).

The reaction was monitored by HPLC analysis of aliquots of the sample solution removed at various times. In runs at elevated temperatures, aliquots of the sample solutions were taken, the reactions were stopped by chilling in an ice bath, and the aliquots were subsequently chromatographed. In the case of reactions at 25°, aliquots were removed and chromatographed directly. Sample volumes used for HPLC were kept small (1–1.5 $\mu$l), since in large volumes the buffers in the reaction mixture were sometimes capable of altering the pH of the mobile phase sufficiently to disturb the chromatographic separation.

To minimize errors due to the possibility of slight variations in the volume of injected samples and the slight changes in instrument sensitivity that appeared to accompany the temperature-dependent fluctuations, the peak areas for III were normalized. The normalization procedure took into account the facts that the reaction involves the hydrolysis of III to yield only I and that the UV detector response to III was only 97% of the response to I on a molar basis. The normalization equation is given in Eq. 1:

$$A_{III,N} = \frac{A_{III,E}}{A_{III,E} + 0.97\,A_{I,E}} \qquad \textbf{(Eq. 1)}$$

where $A_{III,N}$ = normalized area of the peak corresponding to III, $A_{III,E}$ = area of the peak corresponding to III as determined experimentally, and $A_{I,E}$ = area of the peak corresponding to I as determined experimentally. Plots of log $A_{III,N}$ *versus* time were used to calculate the apparent first-order rate constants of the hydrolysis reaction.

*Hydrolysis in Serum and Blood*—Reactions in serum and blood were initiated by adding one volume of a freshly prepared stock solution of III to 10 volumes of either serum or whole blood, both having been previously equilibrated at 37°. After a predetermined time, the protein was precipitated using tungstate-sulfuric acid method of Folin and Wu (16). The precipitant was prechilled to 0° to quench the reaction rapidly. The sample was allowed to stand until coagulation and hemolysis were complete. The samples were then centrifuged, and the supernatant liquid was used for chromatographic analysis.

Such samples did not appear to undergo further reaction to any appreciable extent during the time necessary to complete the separation and analysis. In this case, because of the enzymatic deamination of I in blood (11, 12), normalization as described above was impossible.

**Lyophilization**—An aqueous solution containing ~30 mg of III/ml of water was prepared. Aliquots were placed in suitable screw-capped vials, and these vials were immediately frozen in a freezing mixture of solid carbon dioxide and acetone. The sample vials were then placed in the lyophilizer[14] and freeze dried at a pressure of about 0.2 mm Hg and an initial shelf temperature of from −20 to −25°. The shelf temperature rose to near 0° over 24 hr, during which time most water had been removed. The shelf temperature was then increased to 25°, and the samples were maintained for about 8 more hours at a pressure of about 6.1 mm Hg. The water content of the freeze-dried samples as determined by loss in weight was ~1–2%.

## RESULTS

**Preparation and Characterization**—Compound III was synthesized[15] by dissolving I in anhydrous formic acid (18)[16] and allowing the resulting solution to stand at 5° for 4 days. Isolated yields of III in excess of 50% were obtained. The yield was decreased and the problem of greater quantities of undesired side products was worsened when higher temperatures were used. In addition to III, the 3′-formoyl and the 3′,5′-diformoyl derivatives of I (see *Experimental*) were produced along with a small percentage of adenine (20). The various products were separated by column chromatography and analytically pure III was obtained after recrystallization from acetone.

The characterization of III was based on satisfactory analytical results and NMR spectra. It has been established from studies of model nucleosides and derived esters of known structure that the methylene and methine protons experience deshielding only when attached to the carbon involved in the ester bond. The two methylene (5′) and the three methine (2′, 3′, 4′) protons of arabinosyladenine are represented by two multiplets in the $\delta$ 4.35–3.55 region. In the spectrum of the monoformate (by analysis, $C_{11}H_{13}N_5O_5$), two of those five protons are deshielded; this result is compatible only with Structure III.

**Physical Properties**—The white crystalline prodrug melted at 166–168°, about 90° lower than I. Due to the decomposition of III in water, as discussed later, it was not possible to determine the equilibrium aqueous solubility of the prodrug. However, a concentration of about 34 mg of III/ml of solution was achieved within 1 hr when an amount of the solid in excess of the solubility was agitated with water at 25°. On the basis of these data, it appears that the solubility of III in water is at least 34 mg/ml or nearly 0.12 $M$. This solubility value for III corresponds to the equivalent of ~30.5 mg of I/ml of water, which represents something more than a 60-fold increase in the aqueous molar solubility of III relative to that found for I. This 60-fold increase in the apparent solubility of the drug means that the minimum volume of solution necessary to deliver the equivalent of 2.5 g of I would be reduced from 5 or 6 liters with I itself to about 80 ml with the prodrug.

A comparison of the melting points and the aqueous solubility behavior of I and III clearly demonstrates that formylation of the 5′-position resulted in a significant decrease in intermolecular interaction in the crystalline state. As stated earlier, the qualitative results observed were anticipated on the basis of the hypothesis that acylation of the 5′-hydroxy group would eliminate the ability of that group to act as a hydrogen donor and thereby decrease the extent of intermolecular hydrogen bonding in the solid state. The observed effects on physical properties may arise simply as a direct result of the inability of the 5′-position to act as a proton donor, or they may be due to decreased hydrogen bonding caused by more gross changes in intermolecular and intramolecular geometry in the solid state due to formylation at the 5′-position.

**Hydrolysis in Blood and Serum**—As stated earlier, it was anticipated that the esterases in body fluids would enzymatically catalyze the hydrolysis of the ester prodrug and thereby facilitate rapid and complete reversion to I. The hydrolysis of III at 37° in 91% serum and in 91% whole blood was studied; the data obtained showed that, at an initial concentration of about 2 mg of III/ml of fluid, 50% of the prodrug was lost in about 15–18 min in 91% plasma. However, in 91% whole blood, 50% disappearance of III occurred in about 6–8 min. The observed difference in the time required for disappearance of 50% of the prodrug is probably due to the loss of certain enzymes or enzyme activities, which may occur during the separation of the serum from the whole blood. Observations of this kind have been reported previously for the hydrolysis of aspirin in blood and plasma (21).

More extensive studies in 91% whole blood were performed, and

[13] The thermostated water baths used maintained temperatures at the stated value ±0.2°.

[14] Virtis model 10-800 attached to a Cenco Hyrac 14 vacuum pump.

[15] The synthesis of III was first accomplished in these laboratories utilizing acetic-formic anhydride (17) in formic acid. This method suffered from low overall yields and is, therefore, not presented in detail.

[16] Acylation under acidic conditions has been shown to yield selectively 5′-esters in the case of certain nucleosides (19).

**Table I**—Comparison of the Biological Activity of I and III against Two Experimental Tumors in Mice

| Treatment[a] | Survival Time[b] for Mice Inoculated with Tumor Cells, days | |
|---|---|---|
| | Ehrlich Tumor[c] | Hepatoma 134[d] Tumor |
| Control | 11.6 ± 2.2 | 21.5 ± 1.3 |
| I | 21.0 ± 1.3 | 25.3 ± 2.0 |
| III | 21.0 ± 2.0 | 25.5 ± 1.0 |

[a] I was given at 25 mg/kg ip twice daily on Days 1–4 after inoculation with tumor cells. Compound III was given in equimolar doses according to the same schedule. [b] The values given represent the average survival and the average mean deviation for groups consisting of six mice. [c] ICR female mice were inoculated intraperitoneally with $10^6$ Ehrlich cells. [d] C3H female mice were inoculated intraperitoneally with $5 \times 10^6$ Hepatoma 134 cells.

some typical data are presented in Fig. 1. It was apparent from data of this type that the disappearance of III corresponded quite well to the appearance of I in the initial part of the reaction. However, after about 25% of III had been converted to I, the rates of appearance of I and disappearance of III began to differ markedly and the concentration of I actually reached a maximum and began to decrease prior to the complete disappearance of III. This observation may be explained on the basis of the occurrence of the enzymatic deamination of I to yield the arabinosylhypoxanthine, as previously reported (9, 11) and discussed.

Figure 1 also shows that the apparent rate of hydrolysis of III, when expressed as a percent of the initial concentration, is dependent upon the initial concentration of III. The apparent rate of reversion of III was observed to be more rapid at the lower initial concentration of III, suggesting that the reaction order was less than unity. This observation is probably due to the relatively high concentration of III used, which caused saturation (21) of the esterases involved in the hydrolysis. The initial concentration of 0.4 mg of III/ml of blood represents the range of concentration that would be encountered if a dose of III, which is the equivalent of a 2.0-g dose of I, were administered to an average 70-kg person.

From Fig. 1, it is apparent that even at the lower initial concentration of III, the peak concentration of I approached 50% of the theoretical value. Furthermore, the peak concentration of I expressed as a fraction of the initial dose of III increased as the initial concentration of III was increased; even at the higher initial concentration of III, nearly complete hydrolysis of the prodrug occurred in about 20–25 min.

The positive intercepts of the curves corresponding to the concentration of I at each initial concentration of III are due at least in part to the presence of some I in the aqueous stock solutions prior to the addition of blood.

The data obtained from the blood studies clearly demonstrate that the prodrug rapidly reverts to I and results in a concentration of I that is not currently attainable with I or either of the previously reported prodrug forms (9, 10) in biological fluids.

***In Vivo* Activity**—Subsequent to the *in vitro* studies in blood and serum, the prodrug was compared *in vivo* in both the Ehrlich ascites and Hepatoma 134 rodent tumor systems (Table I)[17]. In both systems, III appeared to have activity equivalent to I when administered intraperitoneally. To obtain meaningful results, evaluation of the prodrug as a method of delivering I should be carried out in systems that possess esterase activity.

***In Vitro* Chemical Stability**—Although the aqueous solubility of III and the *in vitro* and *in vivo* data obtained are such that III would appear to be a prodrug form of I satisfactory for use in intravenous solutions, it was necessary to investigate various other properties and behavior related to stability and formulation before fully evaluating the suitability of III as a prodrug.

As expected, in aqueous buffered solution, III underwent a simple hydrolysis at the 5′-position to yield quantitatively I and formic acid. The reaction was studied at pH ≃ 1–8, and the kinetics of the process appeared to be first order throughout this pH range. The rate of reaction in this region was virtually independent of ionic strength but extremely pH dependent. The log rate–pH profile for hydrolysis of III at 25° is V-shaped (Fig. 2) and is qualitatively similar to that commonly observed for ester hydrolyses, with slopes approaching an absolute value of unity at pH ≤ 2.5 and ≥ 7.0.

[17] These studies were conducted by G. A. LePage, McEachern Laboratory, University of Alberta, Edmonton, Alberta, Canada, who provided the data to the authors.

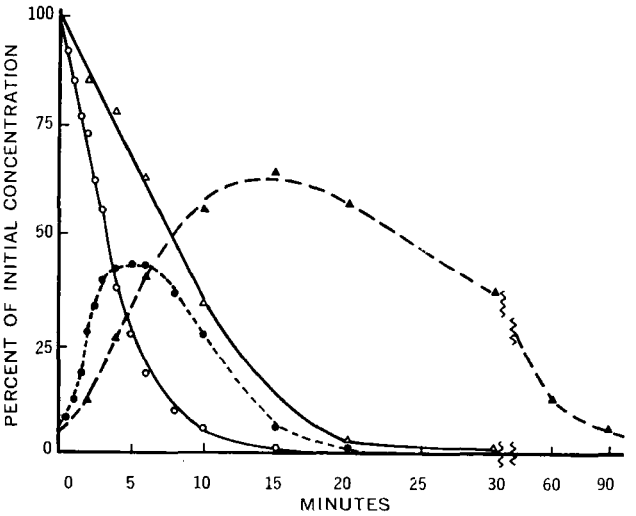



**Figure 1**—*Plot of concentrations of III and I in 91% whole human blood at 37° as a function of time and the initial concentration of III. The triangles represent the concentrations of III (△) and I (▲) at an initial concentration of 2 mg of III/ml of blood solution. The circles represent the concentrations of III (○) and I (●) where the initial concentration of III was 0.4 mg/ml.*

The buffer systems used in the pH range studied were phosphate, chloroacetate, and acetate. All of these buffers were observed to catalyze the ester hydrolyses to a limited extent. Because of such catalysis, the values for the rate constants at the various pH values, as shown in Fig. 2, were obtained by extrapolation to zero buffer concentration of the linear relationship between the observed rate constant and buffer concentration for a given buffer system at that particular pH.

As can be seen from Fig. 2, the minimum spontaneous hydrolyt-

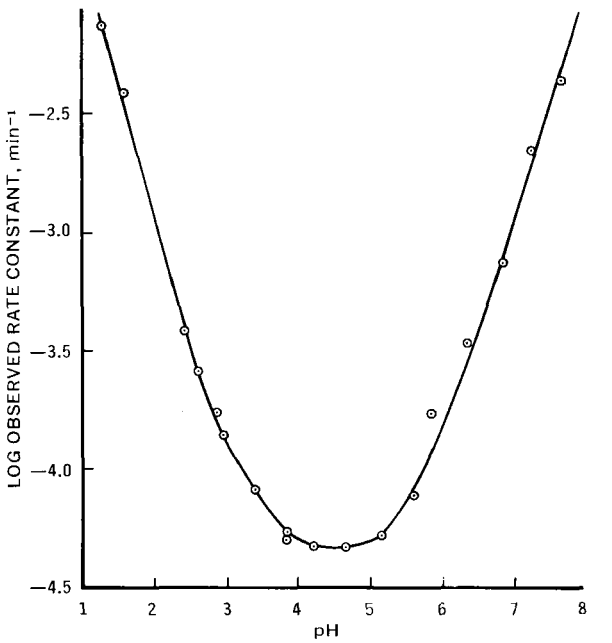



**Figure 2**—*Log rate–pH profile for the spontaneous hydrolysis of III at 25°, ionic strength 0.05. The data points for the various pH values were obtained by extrapolation of plots of observed rates* versus *buffer concentration at each pH.*

**Table II**—Temperature Effect on the Rate of Hydrolysis of III at pH 4.2, Ionic Strength 0.05

| $T$ | $k_0$, $\times 10^4$ min$^{-1a}$ | $t_{1/2}$, days |
|---|---|---|
| 25° | 0.47 | 10.0 |
| 36.8° | 1.4 | 3.4 |
| 60° | 82 | 0.68 |

[a] These values are for the spontaneous hydrolysis of III and were obtained by extrapolation to zero buffer concentration.

ic rate, or the maximum stability of III, is found at pH ~4.2. At this pH the observed first-order rate constant was about $4.7 \times 10^{-5}$ min$^{-1}$ which corresponds to a half-life of about 10 days. At pH 7.4, which approximates physiological pH, the hydrolytic rate constant as shown in Fig. 2 is about $2.5 \times 10^{-2}$ min$^{-1}$ which corresponds to a half-life of about 4.5 hr.

The effects of temperature on the rate of hydrolysis of III at pH 4.2 were determined and these data are presented in Table II. The rate constants were obtained by extrapolation to zero buffer concentration from a series of buffered solutions. The energy of activation for the hydrolytic reaction was calculated from the data in Table I and was found to be about 16 kcal/mole.

**Formulation Considerations**—On the basis of the kinetic data, it was apparent that III would not be suitably stable for formulation as ready-to-use solution even at refrigerated temperatures. However, the 10-day half-life for hydrolysis at pH 4.2 and room temperature appeared to be compatible with its use as a formulation to be reconstituted as a solution, at pH 4.2., within several hours prior to use.

The suitability of the prodrug for use in a lyophilized dosage form was examined in a limited study. An unbuffered aqueous solution containing ~30 mg of III/ml, which is equivalent to ~27 mg of I/ml, was prepared and 1-ml aliquots were quickly frozen and lyophilized. The freeze-drying process resulted in a white porous cake which was readily reconstituted as a solution upon the addition of 1 ml of water. The lyophilization process and subsequent storage of the freeze-dried product in sealed vials for 3 months at 4° resulted in less than 1% degradation of III. These data seem to suggest strongly that formulation of III as a lyophilized dosage form would present no major problems.

## CONCLUSION

On the basis of these results, it appears that III meets virtually all the important criteria for a prodrug form of arabinosyladenine which would be suitable for formulation in parenteral solutions.

More importantly, this work has demonstrated that the design of a prodrug can be accomplished through use of a rational approach. Such an approach requires that the underlying causes which necessitate the use of the prodrug approach be defined and understood. It may then be possible to identify several alternatives which may overcome these basic difficulties. However, only those specific approaches which are compatible with the overall requirements and objectives of the chosen or required pharmaceutical system can be utilized effectively.

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